EXHIBIT A

PHYSIOLOGY OF THE HEART

Fourth Edition

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STRUCTURE OF THE HEART AND CARDIAC MUSCLE

It has been shown by reason and experiment that blood by the beat of the ventricles flows through the lungs and heart and is pumped to the whole body ... the blood in the animal body moves around in a circle continuously, and ... the action or function of the heart is to accomplish this by pumping. This is the only reason for the motion and beat of the heart.

William Harvey
Exercitatio Anatomica de Moto Cordis et Sanguinis in Animalibus, 1628

William Harvey's proof that the heart is a muscular pump overthrew the view, which had dominated European thought for more than 1,000 years, that the heart is the source of the body's heat. Although this made it possible for 18th- and 19th-century anatomical pathologists to understand abnormalities in pump function (Katz, 1997, 1998), it was not until the mid 1950s that efforts to understand the heartbeat began to use new understanding of cell biochemistry and biophysics (Katz and Lorell, 2000). At the end of the 20th century, discoveries in molecular biology made it possible to understand how abnormalities involving specific proteins modify cardiac performance (Katz and Katz, 1991) These findings revealed an elaborate molecular architecture that organizes the heart's electrical activity and maximizes its mechanical efficiency (Katz and Katz, 1989).

ORGAN STRUCTURE

The heart is made up of four pumping chambers: the *right* and *left atria* and the *right* and *left ventricles* (Fig. 1-1). *Atrioventricular valves* lie between the cavities of the atria and ventricles: the *tricuspid valve* on the right and the *mitral valve* on the left. *Semilunar valves*, named for their crescent-shaped cusps, separate each ventricle from its great artery: the *pulmonic valve* between the right ventricle and pulmonary artery and the *aortic valve* between the left ventricle and aorta. All four valves lie in a plane within a connective tissue "skeleton" that separates the atria and ventricles within which the mitral, tricuspid, and
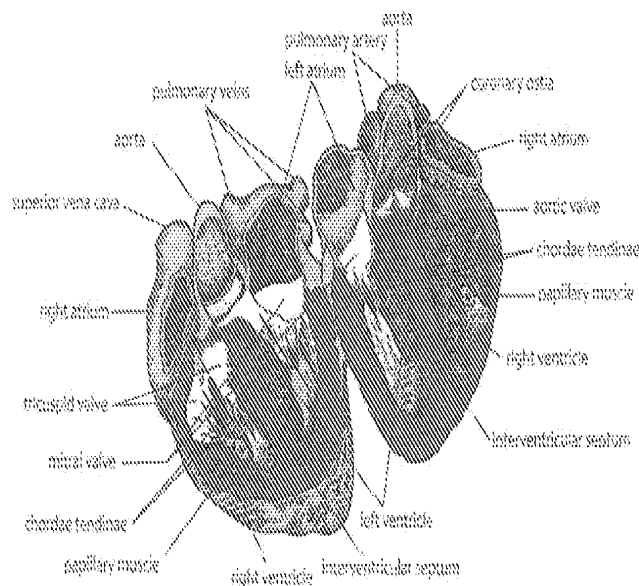
FIGURE 1-1 Major structures in a human heart opened after transection slightly anterior to the midline. (Modified from Berne and Levy, 1967.)

FIGURE 1-2 Schematic diagram of the fibrous skeleton of the heart, viewed from above, showing the 4 valves and the atrioventricular (AV) bundle that crosses this insulating structure through the central fibrous body. Sinuses of Valsalva lie behind the aortic valve cusps, two of which give rise to coronary arteries. The ostium of the left main (LM) lies in the left posterior sinus (LP) while that of the right coronary artery (RCA) lies in the anterior sinus (A); the third sinus of Valsalva, the right posterior (RP), is called the noncoronary sinus because it does not give rise to a coronary artery. The sharper right border of the heart forms the acute margin, the more rounded left border is the obtuse margin.

aortic valves surround a fibrous triangle called the *central fibrous body* (Fig. 1-2). The heart's fibrous skeleton, which can be viewed as a connective tissue "insulator" separating electrically active cardiac myocytes in the atria and ventricles, is penetrated by the *AV (atrioventricular) bundle* (also called the *common bundle* or *bundle of His*), a strand of specialized cardiac muscle that normally provides the only electrical connection between the atria and ventricles. Damage to this critical conducting structure is an important cause of AV block (Chapter 16). When the heart is viewed from the apex, the rounded margin of the left ventricle forms an obtuse angle, whereas the margin of the right ventricle is sharper, like an acute angle; this explains use of the terms *obtuse marginal* and *acute marginal* in naming branches of the coronary arteries (see below).

The semilunar aortic and pulmonary valve cusps are supported by thick tendinous margins. *Sinus of Valsalva* lie behind each of the three aortic valve cusps; the anterior and left posterior sinuses contain the orifices of coronary arteries, while the right posterior, often called the *noncoronary sinus*, does not give rise to a coronary artery (Fig. 1-2). The larger cusps of the mitral and tricuspid valves are tethered at their margins by fibrous *chordae tendinae* that attach to *papillary muscles*, which are "fingers" of myocardium that project into the right and left ventricular cavities (Fig. 1-1). Much as the strands of a parachute arise from a skydiver's harness, several chordae tendinae fan out from each papillary muscle to the valve margins. Laxity of the connective tissue supporting the mitral valve can allow the leaflets to move backward (prolapse) into the atria when intraventricular pressure rises during systole (Becker and

deWit, 1979). The abnormal valve opening, which can cause an audible "click," may permit blood to leak into the left atrium (mitral regurgitation), causing a late systolic murmur. This syndrome, called *mitral valve prolapse*, is often of no clinical significance, but when caused by connective tissue abnormalities, significant mitral regurgitation can occur. Rupture of a papillary muscle, which sometimes occurs after myocardial infarction (see Chapter 17), causes severe mitral regurgitation and is often fatal.

Architecture of the Walls of the Heart

The thin-walled atria, which develop relatively low pressures, contain ridges of myocardium called *pectinate muscles* that may represent preferential conducting pathways linking the sinoatrial (SA) and AV nodes; these are sometimes referred to as *internodal tracts or sinoatrial ring bundles* (Hayashi et al., 1982; Anderson and Ho, 1998). The ventricles develop much higher pressures than the atria and therefore have thicker muscular walls. The left ventricle, which has approximately three times the mass and twice the thickness of the right ventricle, can be viewed as a "pressure pump" whose cavity resembles an elongated cone in which inflow and outflow tracts lie side-to-side in the wider end (Fig. 1-3). The right ventricle, which pumps at lower pressure and operates as a

FIGURE 1-3 A: Schematic anterior views of the right and left ventricular chambers. In the U-shaped right ventricle, the inflow (tricuspid) and outflow (pulmonic) valves are widely separated, whereas in the conical left ventricle, the mitral and aortic valves lie side-by-side, where they are separated by the anterior leaflet of the mitral valve. B: Casts of canine right and left ventricular cavities. Left: Anterior view. Right: Superior view showing approximate locations of the pulmonic (PV) and tricuspid (TV) valves in the right ventricle, and the aortic (AV) and mitral (MV) valves in the left ventricle.
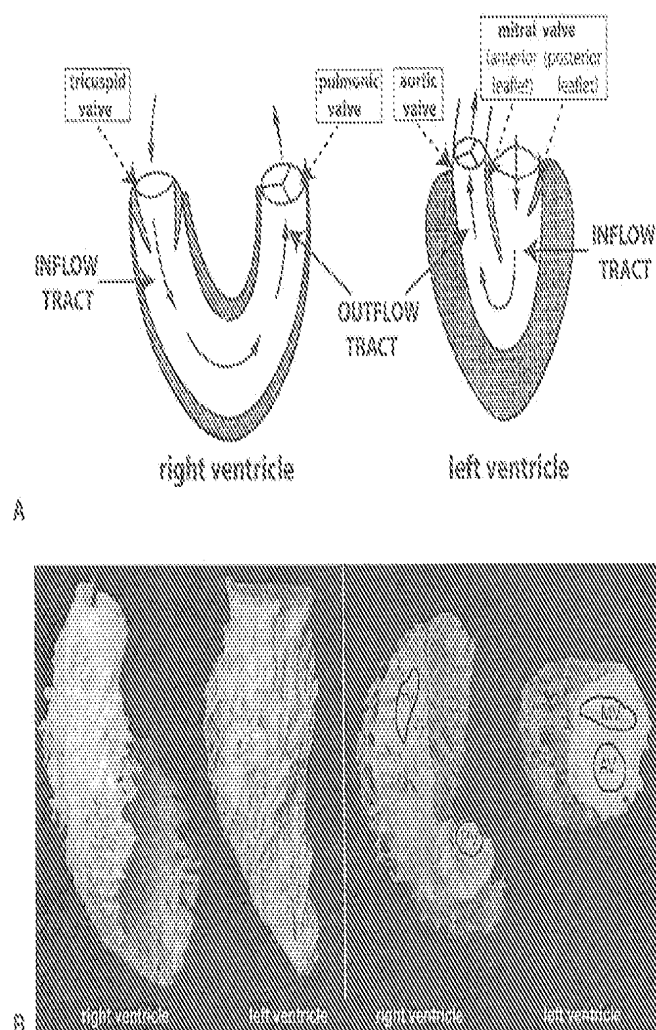
"volume pump," is shaped like a crescent with inflow through the tricuspid valve at one end and outflow through the pulmonic valve at the other (Fig. 1-3). During systole, the interventricular septum normally moves toward the left ventricular free wall and participates in left ventricular ejection. In chronic right ventricular overload—for example, in patients with pulmonary hypertension—the septum can move paradoxically away from the left ventricular cavity during systole to aid right ventricular ejection.

The heart, along with a small amount of fluid, is contained within a noncompliant fibrous sac called the *pericardium* whose inner surface, the *parietal pericardium*, is continuous with the *epicardium*, a layer of connective tissue that covers the outer surface of the heart. The cavities of the atria and ventricles, along with the valves, are lined with another connective tissue layer called the *endocardium* (Brutsaert, 1989). Because the heart is contained within the rigid pericardium (see below), the ventricles interact with one another. These interactions are especially important in diastole, when dilatation of one ventricle can impair the filling of the other (Yacoub, 1995; Santamore and Dell'Italia, 1998; Morris-Thurgood and Frenneaux, 2000).

The left ventricle, which is conical in shape during diastole, becomes more spherical as intraventricular pressure rises during systole (Hawthorne, 1969). Ejection propels blood superiorly (toward the head) so that—according to Newton's Law, *for every action there is an equal and opposite reaction*—the base of the heart moves inferiorly (toward the feet). This movement, called *descent of the base*, explains the prominent "x descent" in the normal venous pulse.

The muscular walls of the ventricles are made up of overlapping sheets—sometimes called *bulbospiral* and *sinuspiral* muscles—that follow spiral paths as they sweep from the fibrous skeleton at the base of the heart to its apex (Fig. 1-4) (Grant, 1965). The muscle fibers at the epicardial surface of the left ventricle tend to parallel the base-apex axis of the heart, whereas those at the endocardial surface are oriented more circumferentially (Streeter et al., 1969) (Fig. 1-5). During systole, as the ventricles empty, these muscle bundles thicken but undergo little angular distortion (Fenton et al., 1978).

Electrical Activation

The heartbeat is initiated and controlled by electrical impulses that are generated and conducted by specialized myocardial cells (Chapter 15). Activation normally begins in the *SA node* (Fig. 1-6), a band of spontaneously depolarizing cells that lies between the superior vena cava and right atrium (Oosthoek et al., 1993b; Verheijck et al., 1998; Anderson and Ho, 1998). Because of its rapid firing rate, the SA node, which is derived from the embryonic right sinus venosus, normally serves as the heart's pacemaker.

The wave of depolarization initiated by the SA node is propagated through atrial myocardium, first to the right atrium and then to the left atrium. After encountering a delay in the *AV node*, which is derived from the embryonic left sinus venosus, the wave of depolarization enters the ventricles through the *AV bundle* (Oosthoek et al., 1993a), which bifurcates at the top of the interventricular septum into *right and left bundle branches*. The right bundle branch crosses the right ventricular cavity within the *moderator band*, a muscular bundle that extends from the interventricular septum to the base of the papillary muscle, which supports the anterior leaflet of the tricuspid valve (Fig. 1-6). The left bundle branch is often stated to bifurcate into *anterior* and *posterior fascicles*,
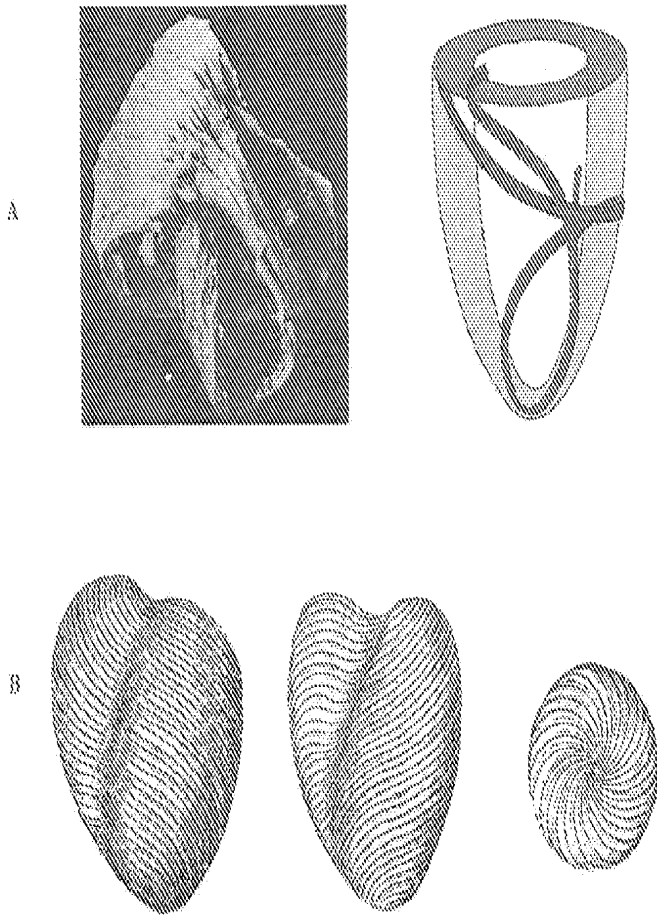
FIGURE 1-4 Spiral musculature of the ventricular walls. A: Spiral bundles in a dissected human heart sweep from the fibrous skeleton at the base of the heart (above, left) to the apex (below). (From Grant RP, 1965, by permission of the American Heart Association.) B: Schematic drawing of spiral bundles in the left ventricle. (Modified from Lower, 1669.)

but as discussed in Chapter 15, this is generally an oversimplification. Impulses transmitted via the bundle branches then enter the *His-Purkinje system*, a subendocardial network of rapidly conducting cells that synchronizes ventricular activation. The electrophysiological properties of myocardial cells in different layers of the ventricular wall are not the same;

EPICARDIUM   ENDOCARDIUM
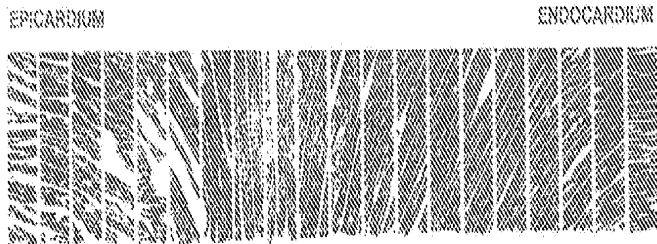
FIGURE 1-5 Reconstruction of the left ventricular wall, prepared from a series of microphotographs, showing different fiber angles between the epicardium (left) and endocardium (right). (Modified from Streeter et al., 1969, by permission of the American Heart Association.)
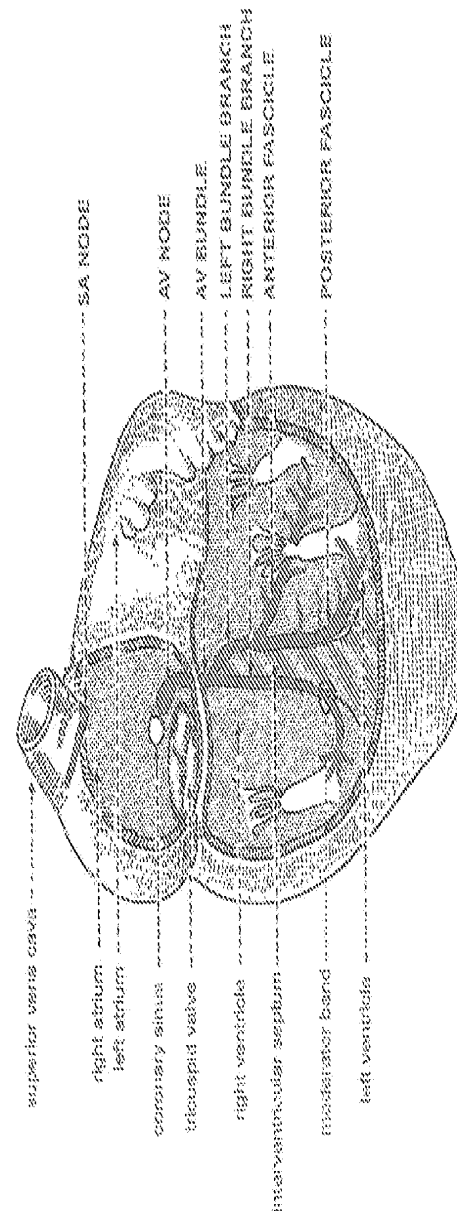
FIGURE 1-6 Conducting system of the human heart (capitalized labels at right) and major anatomical features (lowercase labels at left). (Modified from Benninghoff, 1944.)

for example, action potential duration in the endocardium is longer than that in the epicardium and is longest in *M-cells* found in the mid-regions of the ventricular wall (see Chapter 14).

THE CORONARY CIRCULATION

Major Epicardial Coronary Arteries

Large epicardial coronary arteries carry virtually all of the blood that supplies the heart. Although a few layers of endocardial myocytes are perfused from the ventricular cavities via *arteriosinusoidal* and *arterioluminal vessels*, this auxiliary blood supply is of no practical importance when a large coronary artery becomes occluded (Chapter 17). The major coronary arteries are abbreviated *LEFT MAIN* (left main coronary artery), *RCA* (right coronary artery), *LAD* (left anterior descending), *CIRC* (circumflex), and *PDA* (posterior descending) (Fig. 1-7). All lie in grooves between the heart's chambers—the RCA and CIRC between the atria and ventricles, the LAD and PDA between the left and right ventricles.

The anatomy of these vessels can be summarized by the statement *"three out of two makes four."* Two coronary arteries arise from the aorta

(RCA and LEFT MAIN) and continue after the LEFT MAIN divides into the LAD and CIRC as *three* vessels (RCA, LAD, and CIRC). After the PDA arises from either the RCA or CIRC, the heart is supplied by *four* large arteries (RCA, LAD, CIRC, and PDA).

The LEFT MAIN, which originates in the left posterior sinus of Valsalva (Fig. 1-2), continues as a single vessel of variable length before dividing into two major branches—the LAD and CIRC (Fig. 1-7). The LAD, which courses down the anterior interventricular groove, gives rise to *septal perforating arteries* that supply the anterior two-thirds of the interventricular septum, *diagonal branches* that supply the anterior wall of the left ventricle, and *right ventricular branches* that provide blood to the anterior wall of the right ventricle. After crossing the apex of the heart, the LAD usually turns upward to run a short distance toward the base in the posterior interventricular groove (Figs. 1-7 and 1-8). The CIRC, which courses to the left in the anterior atrioventricular groove, gives rise to *obtuse marginal branches* that supply the lateral wall of the left ventricle. The PDA, which runs inferiorly toward the apex in the posterior interventricular groove, can be supplied by the CIRC or the RCA. In most human hearts, the CIRC, after reaching the back of the
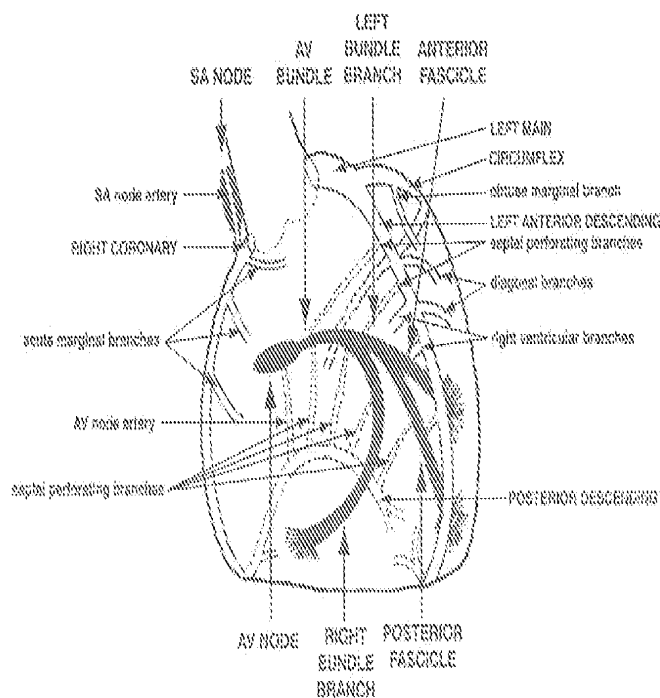
FIGURE 1-7 Major coronary arteries and their branches (labels at right and left) and key elements of the cardiac conduction system (labels above and below). AV, atrioventricular; SA, sinoatrial.

A. Left dominant   **B. Right dominant**
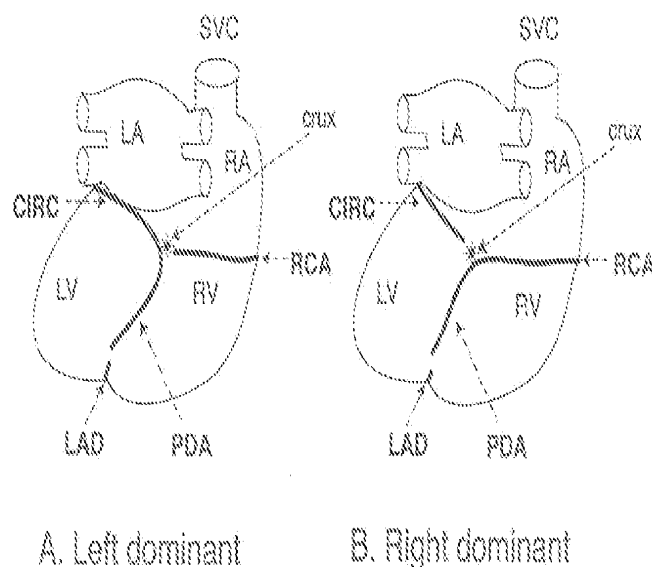
FIGURE 1-8 Posterior view of the human heart showing left dominant (A) and right dominant (B) coronary artery distribution. In the left dominant, the posterior descending artery (PDA) is a continuation of the circumflex branch of the left coronary artery (CIRC) that runs from the crux of the heart down the posterior interventricular groove; more commonly, in the right dominant distribution, the posterior descending artery is a continuation of the right coronary artery (RCA). The left anterior descending coronary artery (LAD), after wrapping around the inferior surface of the heart, usually courses upward for a short distance in the posterior interventricular groove. LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle; SVC, superior vena cava.

left ventricle, runs only a short distance down the posterior interventricular groove to end near the *crux of the heart*, where the plane of the interventricular septum crosses the plane of the atrioventricular groove (Fig. 1-8B). This distribution, in which the PDA is supplied by the RCA, is called *right dominant* and occurs in approximately 90% of human hearts. In the remaining 10% or so, the CIRC turns downward at the crux to supply the PDA (*left dominant*, Fig. 1-8A).

The RCA, which arises from the anterior aortic sinus, courses toward the right in the anterior atrioventricular groove, where it gives rise to *right ventricular (acute marginal) branches* that supply the free wall of the right ventricle. The RCA then crosses the acute margin of the heart, turns to the left in the posterior atrioventricular groove, and after reaching the *crux of the heart* usually continues in the posterior interventricular groove as the PDA ("right dominant" coronary circulation, Fig. 1-8B).

As already noted, the PDA, which supplies *septal perforating branches* that perfuse the posterior third of the interventricular septum, arises from the RCA in approximately 90% of human hearts and from the CIRC in about 10%.

Coronary atherosclerosis is often described as *one-vessel*, *two-vessel*, and *three-vessel* disease, terms that describe how many of the three major arteries (RCA, LAD, and CIRC) are narrowed. Obviously, the more vessels that are occluded, the more severe is the coronary disease. LEFT MAIN disease is especially dangerous because this vessel supplies both of the arteries that supply blood to the left ventricle (LAD and CIRC).

Collateral Vessels

The coronary arteries in humans can be viewed as "end-arteries" because there is little flow between the vascular beds supplied by the different major arteries (Factor et al., 1981). Collateral vessels, which connect arterial systems supplied by different major epicardial arteries, are not found at the level of the microcirculation, so that occlusion of a major epicardial artery generally causes an infarct whose borders are sharply demarcated from the adjacent myocardium supplied by other, nonoccluded arteries. Collateral vessels can connect larger arteries, but these are poorly developed in young individuals. However, in patients with chronic coronary atherosclerosis, these collaterals often enlarge and can play an important role in maintaining blood flow to the heart after a major coronary artery is occluded.

Blood Supply to the Ventricular Myocardium

The devastating consequences of coronary occlusion reflect the fact that cardiac contraction depends on an uninterrupted delivery of oxygen (Chapter 2). Oxygenated blood from the epicardial arteries reaches the
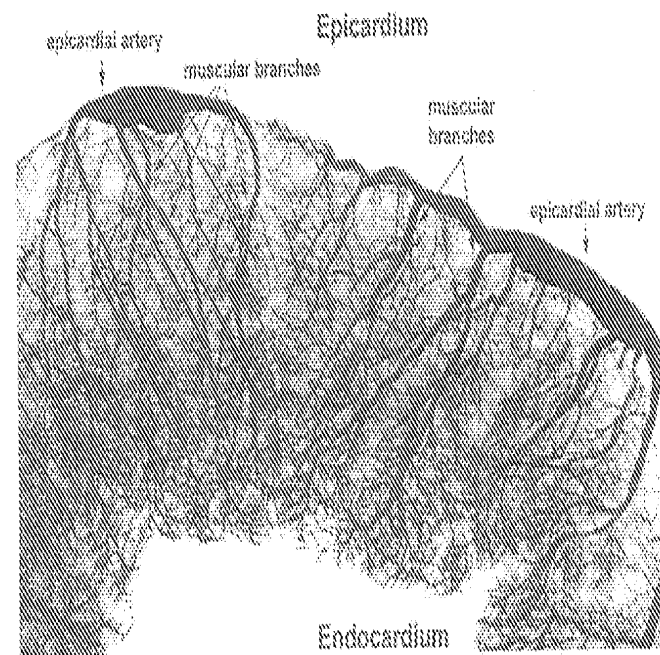
FIGURE 1-9 X-ray microphotograph of a heart injected with radiopaque dye showing muscular branches of large coronary arteries that penetrate the myocardium from the epicardial surface of the ventricle. (Modified from Schäper, 1979.)

myocardium via muscular branches that traverse the walls of the ventricles (Fig. 1-9). Because intramyocardial pressure compresses these vessels, the subendocardial regions of the thick-walled left ventricle are especially vulnerable to coronary artery narrowing. Normal compression of the muscular branches during systole explains why virtually all nutrient coronary flow occurs during diastole.

The *left ventricular papillary muscles* receive their blood supply from large penetrating vessels called *perforators*. The *anterolateral papillary muscle*, which supports the anterior leaflet of the mitral valve, has a dual blood supply derived from branches of the CIRC and LAD. The *posteromedial papillary muscle*, which supports the posterior leaflet, receives its blood supply from the PDA, and thus from the RCA, in the majority of human hearts that are "right dominant," and from the CIRC in those with "left dominant" coronary circulation (see above).

Blood Supply to the Conduction System

The SA node is perfused by the *SA node artery* (Fig. 1-7), which in slightly more than half of human hearts is a branch of the RCA; in the remainder, this artery arises from the CIRC. The AV node is usually supplied by an *AV node artery* that is a branch of the PDA; the blood supply to the AV node is therefore derived from the RCA in about 90% of human hearts and the CIRC in approximately 10%.

The AV bundle, along with proximal portions of both right and left bundle branches, is perfused by *septal perforators* that arise from both the LAD and PDA. Because this critical structure has a dual blood supply, damage to the AV bundle in ischemic heart disease implies that more than one major coronary artery is occluded. The anterior division of the left bundle branch and mid-portion of the right bundle branch are supplied by septal perforators arising from the LAD, while the posterior division of the left bundle branch is perfused by septal perforators supplied by the PDA.

Coronary Venous Drainage

The venous effluent of the heart is collected in veins that parallel the epicardial coronary arteries. Most venous drainage of the left ventricle enters the *coronary sinus*, which parallels the CIRC in the left posterior atrioventricular groove before emptying into the floor of the right atrium. A small portion of the venous drainage of the left ventricle, along with much of that derived from the right ventricle, enters the right atrium through *anterior cardiac veins*. A minor fraction of the venous drainage of the ventricular myocardium drains directly into the cavities of the right and left ventricles by way of *Thebesian veins*.

The ostium of the coronary sinus provides a landmark for the AV node, which lies immediately above this opening. Because the coronary sinus passes to the left behind the heart in the left atrioventricular groove, specially designed catheters can be used to record the electrical activity of the left atrium and ventricle, stimulate these structures, and perform ablation therapy on the left side of the heart.

FRACTAL ANATOMY OF THE HEART

Many asymmetrical structures in the heart, including the coronary blood vessels, chordae tendineae, and interventricular conduction system, form networks whose outwardly disorganized branching follows complex rules. These are described mathematically as *fractals*, which describe the order often found in seemingly random biological structures (Goldberger et al., 1990) and functional disorders (Goldberger et al., 2002).

LYMPHATICS

The *lymphatic vessels* that drain the heart's interstitium run alongside the coronary arteries and veins in the atrioventricular and interventricular grooves. Most cardiac lymphatic channels cross the anterior surface of the pulmonary artery to reach *pretracheal lymph nodes* that drain through a *cardiac lymph node* situated between the superior vena cava and right innominate artery. The lymph ultimately drains into the thoracic duct (Miller, 1982).

INNERVATION

The heart is richly innervated by both *sympathetic* and *parasympathetic* nerves. Postganglionic sympathetic fibers arise mainly in the fourth and fifth thoracic segments of the spinal cord and form synaptic connections in the cervical and thoracic cervical ganglia (often called *stellate ganglia*) and cardiac plexus. Postsynaptic sympathetic nerves do not form specialized junctions in the heart but instead release their neurotransmitter (norepinephrine) from varicosities that lie in plasma membrane depressions on the surface of cardiac myocytes. The heart's parasympathetic innervation originates in the dorsal efferent nuclei of the medulla oblongata and reach the heart by way of the cardiac branches of the vagus nerve. Preganglionic parasympathetic fibers impinge on postganglionic cells in the SA and AV nodes, the atria, and the heart's blood vessels, but parasympathetic innervation of the ventricular myocardium is more limited.

Sensory fibers that originate in the heart reach the brain stem by way of the cardiac plexus. Stimulation of these fibers informs patients when their heart becomes energy starved. A chest discomfort called *angina pectoris* is the most common perception caused by cardiac ischemia.

Stretch receptors located in the inferior and posterior walls of the left ventricle can evoke a powerful vagal response called the *von Bezold-Jarisch reflex* (Dawes and Comroe, 1954). This reflex, which is often activated in inferior and posterior wall myocardial infarction, can slow the SA node, inhibit conduction through the AV node, and cause blood pressure to fall (see Chapter 17).

HISTOLOGY

The outer surfaces of the atria and ventricles are covered by a layer of squamous cells and a network of fibroelastic connective tissue called the *epicardium*. The *endocardium*, which lines the heart's chambers, is also made up of squamous cells beneath which is a mesh of collagen and elastic fibers and a rudimentary layer of smooth muscle. The *myocardium*, which makes up the vast majority of the heart's thickness, contains both myocytes and connective tissue. Although cardiac myocytes represent most of the myocardial mass, approximately 70% of the cells are smaller nonmyocytes that include vascular smooth muscle, endothelial cells, and fibroblasts. The latter secrete and maintain the connective tissue fibers that contribute to the heart's tensile strength and stiffness. This connective tissue framework is organized into the *endomysium*, which surrounds individual cardiac myocytes; the *perimysium*, which supports groups of myocytes; and the *epimysium*, which encases the entire muscle (Fig. 1-10).

Several types of cardiac myocytes are found in the adult human heart (Fig. 1-11). The most numerous are *working myocytes* of the atria and ventricles that are specialized for contraction. Atrial myocytes are smaller
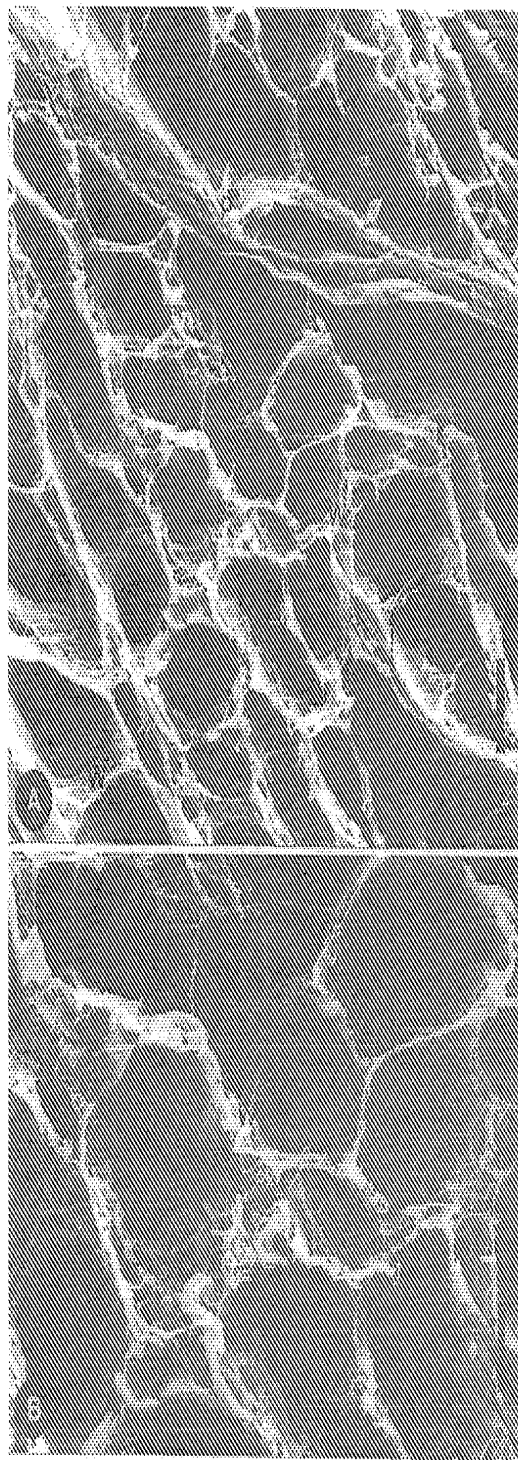
FIGURE 1-10 Connective tissue framework of the human heart, showing groups of myocytes surrounded by the perimysium (P). A weave of endomysium that surrounds the individual myocytes (M) forms lateral struts (S) that connect adjacent cells. Collagen struts also connect myocytes to microvessels (thin arrow) and to the perimysium (thick arrow). (From Rossi et al., 1998, by permission of the American Heart Association.)
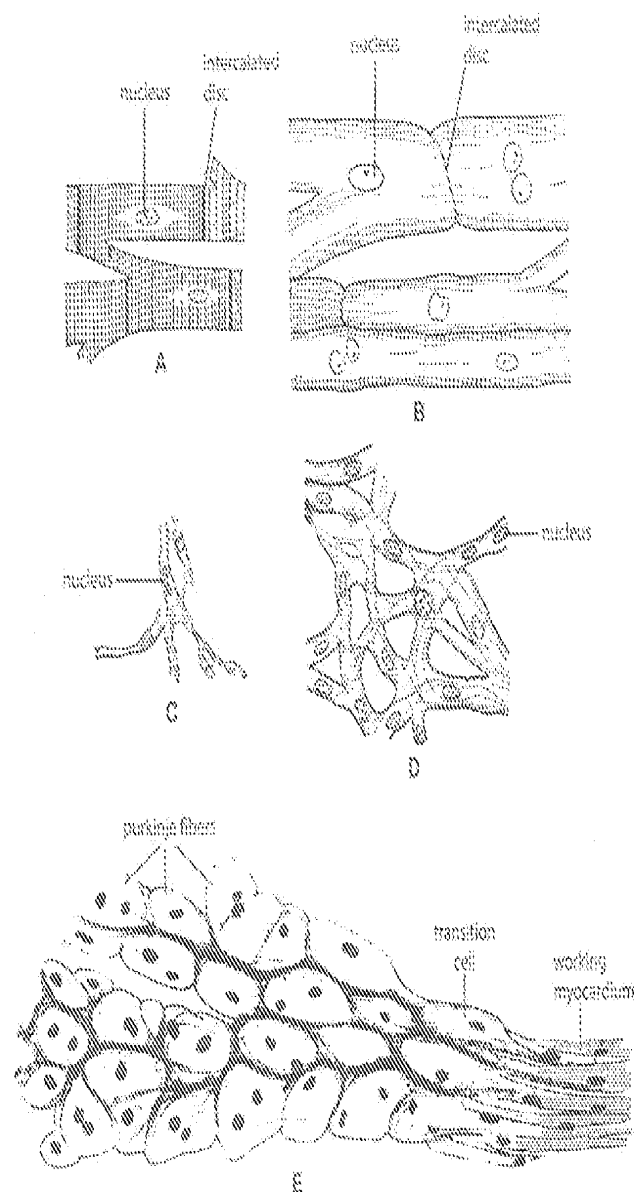
FIGURE 1-11 Human cardiac myocytes. A: Working ventricular myocytes contain cross striations, central nuclei, and intercalated discs. B: Purkinje fibers are large, poorly stain-ing cells with sparse cross striations. The SA node (C) and AV node (D) are networks of small, sparsely cross-striated cells. E: Transition cells are seen where Purkinje fibers (left) impinge on the working myocardium (right). (Modified from Benninghoff, 1944.)

in diameter than those of the ventricles. *Purkinje fibers*—found in the AV bundle, bundle branches, and ventricular endocardium—are specialized for rapid conduction. *Nodal cells* in the SA and AV nodes are responsible for pacemaker activity and an atrioventricular conduction delay, respec-tively. Additional heterogeneity is seen at the molecular level, where his-tologically similar cardiac myocytes represent different molecular pheno-types that are distributed in a mosaic pattern (Fig. 1-12) (Sartore et al., 1981; Bouvagnet et al., 1984).
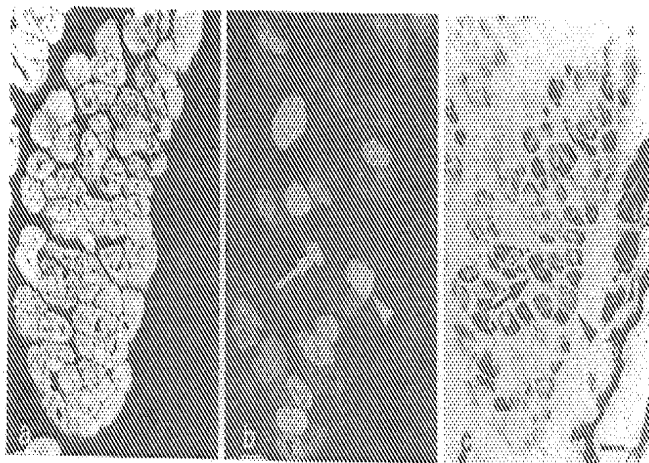
FIGURE 1-12 Microphotographs of serial sections from human right atrium incubated with two different immunofluorescent antiventricular human myosin antibodies (a, b) and stained histochemically for myosin ATPase activity (c). One antibody binds to all atrial myosin isoforms (a), the other binds more strongly to some cells (b). The arrowheads in (b) and (c) show a fiber that binds the antibody used (b) but exhibits weak ATPase activity in (c), while the arrows show a cell that binds weakly to the antibody but exhibits high ATPase activity. Bar = 20 μM. (Reprinted from Bouvagnet et al., 1984, by permission of the American Heart Association.)

The cells of the myocardium are arranged in a branched network that was once believed to represent an anatomical syncytium. However, the *intercalated discs*, which are densely staining transverse bands that characteristically appear at right angles to the long axis of the cardiac myofibers, are now known to represent specialized cell-cell junctions that contain regions of low electrical resistance (see below). While not a true anatomical syncytium, the heart functions as if all of the myocytes are in free electrical communication.

Working cardiac myocytes, which usually contain a single centrally located nucleus, are filled with cross-striated myofibers and mitochondria (Fig. 1-11A). *Purkinje fibers*, which are specialized for rapid conduction, are large pale cells that contain more glycogen but fewer contractile filaments and mitochondria (Fig. 1-11B). Cells intermediate in appearance between the Purkinje fibers and the working cardiac myocytes are called *transition cells* (Fig. 1-11E). The myocytes in the *SA node* (Fig. 1-11C) and *AV node* (Fig. 1-11D), like Purkinje fibers, are rich in glycogen and contain few contractile filaments; however, nodal cells conduct slowly because of their small size and high internal electrical resistance. Unlike the working myocytes of the atria and ventricles, which use oxidative reactions to generate ATP, the myocytes that make up the heart's conduction system rely on anaerobic energy production (Henry and Lowry, 1983).

Atrial cardiac myocytes contain granules that represent stores of the biologically active *atrial natriuretic peptide* (ANP), which is natriuretic and diuretic and relaxes vascular smooth muscle. The heart is therefore not only a pump, but also an endocrine organ. Small amounts of ANP and the structurally related *brain natriuretic peptide* (BNP) also are found in the ventricles. These peptides are released when the walls of the heart are stretched and can be viewed as "volume sensors" that help the body to defend against expanded blood volume (for a review, see Levin et al., 1998; Boomsma and Van der Meiracker, 2001).

ULTRASTRUCTURE

The *contractile proteins*, which make up almost one-half of the volume of working cardiac myocytes, are organized in a regular array of cross-striated myofibrils (Figs. 1-13 and 1-14). Most of the remaining cell volume is occupied by *mitochondria* that generate the large amounts of chemical energy required for contraction (Table 1-1). Key membrane systems that regulate cardiac performance include the *plasma membrane*, which separates the cytosol from the surrounding extracellular space, and the intracellular membranes of the *sarcoplasmic reticulum* (Table 1-2). The most abundant membranes are those of the mitochondria (Page, 1978).

Myofibrils

The cross-striated pattern in working cardiac myocytes (Figs. 1-13–1-15) reflects the distribution of contractile protein filaments. The more darkly staining striations, which contain a parallel array of thick filaments (see below), strongly rotate polarized light and are anisotropic (birefringent), hence their designation *A-bands*. The more lightly staining striations, which contain only thin filaments, are the more isotropic (less birefringent) *I-bands*. Each I-band is bisected by a darkly staining *Z-line*. The fundamental morphological unit of striated muscle is the *sarcomere*, which is defined as the region between two Z-lines; each sarcomere therefore includes a central A-band and two adjacent half I-bands.

The thick filaments that extend the length of the A-band are polymers of *myosin* and a huge protein called *titin*. The central regions of the thick filaments also contain *myosin-binding protein C, M-protein, myomesin*, and the MM isoform of *creatine phosphokinase* (see Chapter 5). *Cross-bridges* that project from the thick filaments and interact with the thin filaments represent the heads of myosin molecules. The thin filaments are double-stranded *actin* polymers that include *tropomyosin* and the three proteins of the *troponin complex* (see Chapter 4). At the Z-lines, the thin filaments are interwoven with several cytoskeletal proteins, including *α-actinin, Cap Z (β-actinin), nebulette*, and *desmin*, which
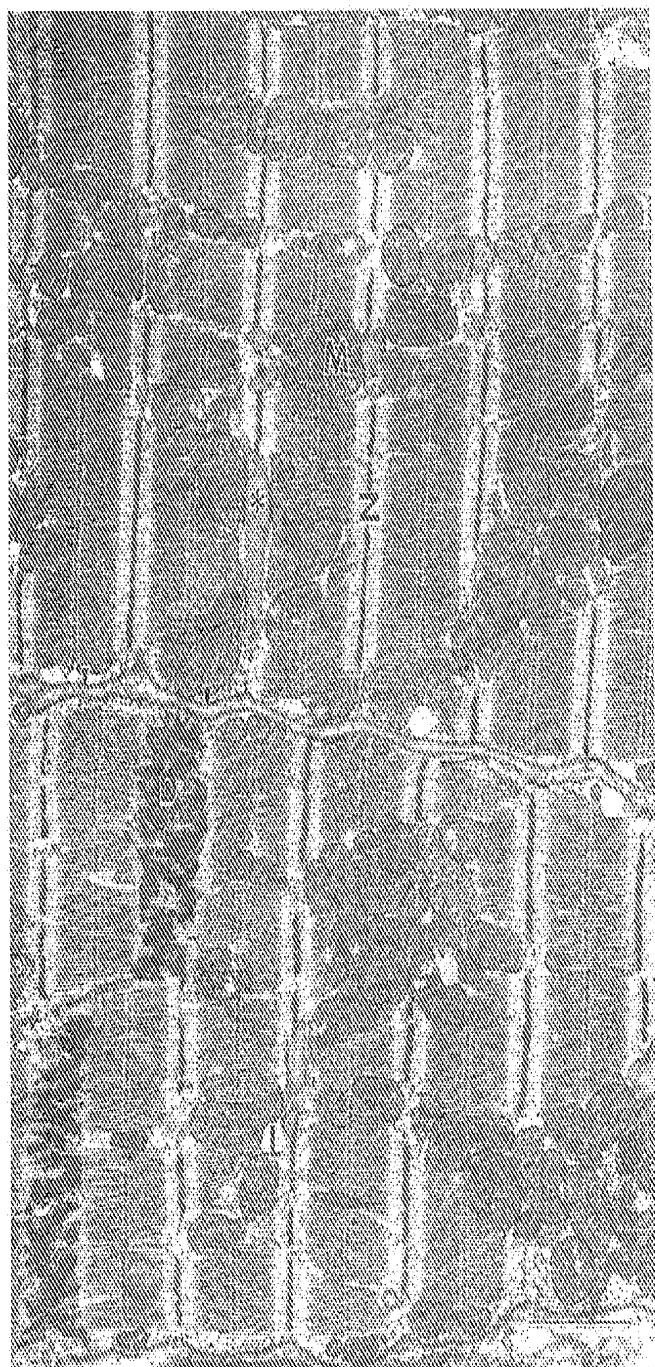
FIGURE 1-13 Electron microphotograph of two normal human left ventricular myocytes (above and below) that are separated by a thin band of extracellular fluid (oriented from right to left in center of figure). Sarcomeres are aligned within each cell. Endomysium separates the cells (arrowheads). M, mitochondria; Z, Z-lines; D, intercalated disc; L, lipid droplet; *, t-tubule. Scale bar = 2 μM. (Reproduced with permission from Gerdes et al., 1995.)
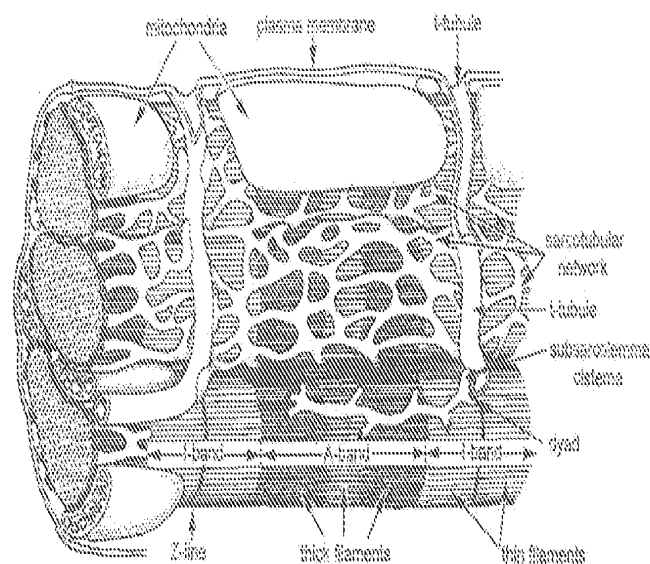
FIGURE 1-14 Ultrastructure of a working cardiac myocyte. Contractile proteins are arranged in a regular array of thick and thin filaments (seen in cross section at left). The A-band represents the region of the sarcomere occupied by the thick filaments into which thin filaments extend from either side. The I-band contains only thin filaments that extend toward the center of the sarcomere from Z-lines that bisect each I-band. The sarcomere, the functional unit of the contractile apparatus, lies between two Z-lines and contains one A-band and two half I-bands. The sarcoplasmic reticulum, an intracellular membrane system that surrounds the contractile proteins, consists of the sarcotubular network at the center of the sarcomere and subsarcolemmal cisternae. The latter form specialized composite structures with the transverse tubular system (t-tubules) called dyads. The t-tubular membrane is continuous with the sarcolemma; the lumen of the t-tubules contains extracellular fluid. Mitochondria are shown in the central sarcomere and in cross section at left. (Modified from Katz, 1975.)

TABLE 1-1 Morphology of a Working Myocardial Cell (Rat Left Ventricle)

Component	Percent of Cell Volume
Myofibrils	47
Mitochondria	36
Sarcoplasmic reticulum	3.5
Subsarcolemmal cisternae	0.35
Sarcotubular network	3.15
Nuclei	2
Other (mainly cytosol)	11.5

Modified from Page, 1978.

TABLE 1-2 Membrane Areas in a Working Myocardial Cell (Rat Left Ventricle)

Membrane	μm^2 Membrane Area per μm^3 Cell Volume
Plasma membrane	0.465
Sarcolemma	0.31
t-Tubules	0.15
Nexus	0.005
Total sarcoplasmic reticulum	1.22
Subsarcolemmal cisternae	0.19
Sarcotubular network	1.03
Mitochondria	20

Modified from Page, 1978.

attach the sarcomeres to cell adhesion molecules that link myocytes to each other and to the extracellular matrix (see Chapter 5).

The lengths of the thick and thin filaments remain constant during contraction and relaxation, so that changes in the extent of overlap between thick and thin filaments cause sarcomeres to shorten and lengthen (Fig. 1-16). During systole, changes in the orientation of the myosin cross-bridges

FIGURE 1-16 Schematic diagram of a sarcomere showing length-dependent changes in the overlap between thick and thin filaments. A: At long sarcomere lengths in resting muscle (note myosin cross-bridges at right angles to the thick filament), the thin filaments do not extend to the center of the A-band. B. During contraction, the thin filaments are drawn toward the center of the sarcomere (note angulated myosin cross-bridges attached to thin filaments). C: As the sarcomere shortens further, the thin filaments of adjacent I-bands pass in the center of the A-band ("double overlap").

draw the thin filaments toward the center of the A-band (see Chapter 4). At very short sarcomere lengths, thin filaments from the two sides of the sarcomere pass in the center of the A-band, giving rise to "double overlap" (Fig. 1-16).

In cross section, the A-band is a hexagonal array of thick filaments, each of which is surrounded by six thin filaments that lie at the trigonal points between adjacent thick filaments (Figs. 1-17 and 1-18).

FIGURE 1-15 Electron microphotograph of a sarcomere in normal human left ventricle. A grazing section on the left side of the sarcomere shows the sarcotubular network (S) overlying the I-band. Mitochondria are seen above the sarcomere. M, line in the center of the A-band (A); I, I-band; Z, Z-line; T, t-tubule. Scale bar = 2 μM. (Reproduced with permission from Gerdes et al., 1995.)

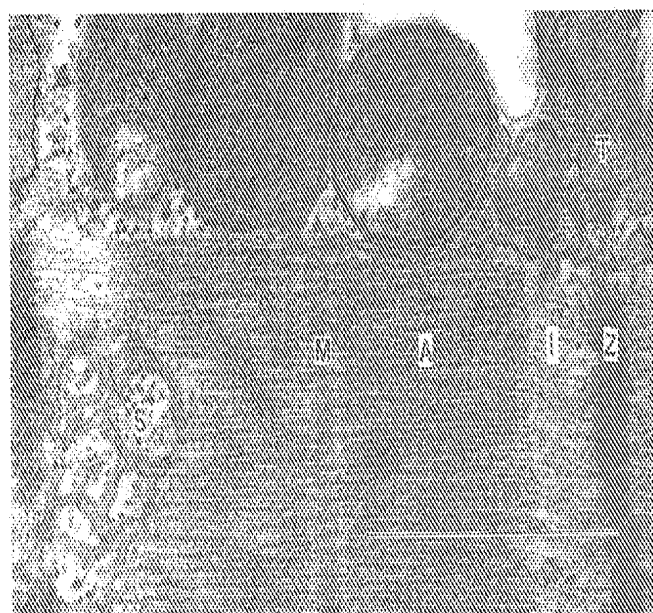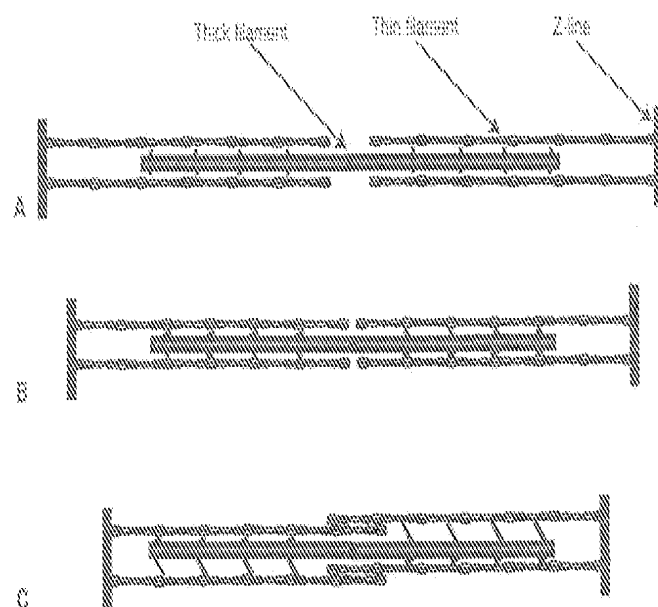FIGURE 1-17 Schematic cross sections at different levels of the sarcomere. A: In the A-band, thin filaments lie at the trigonal points in a hexagonal array of thick filaments. I: In the I-band, where thick filaments are absent, the thin filaments are less ordered. M: Thin radial filaments made up of myosin-binding protein C in the M-band at the center of the A-band connect adjacent thick filaments.
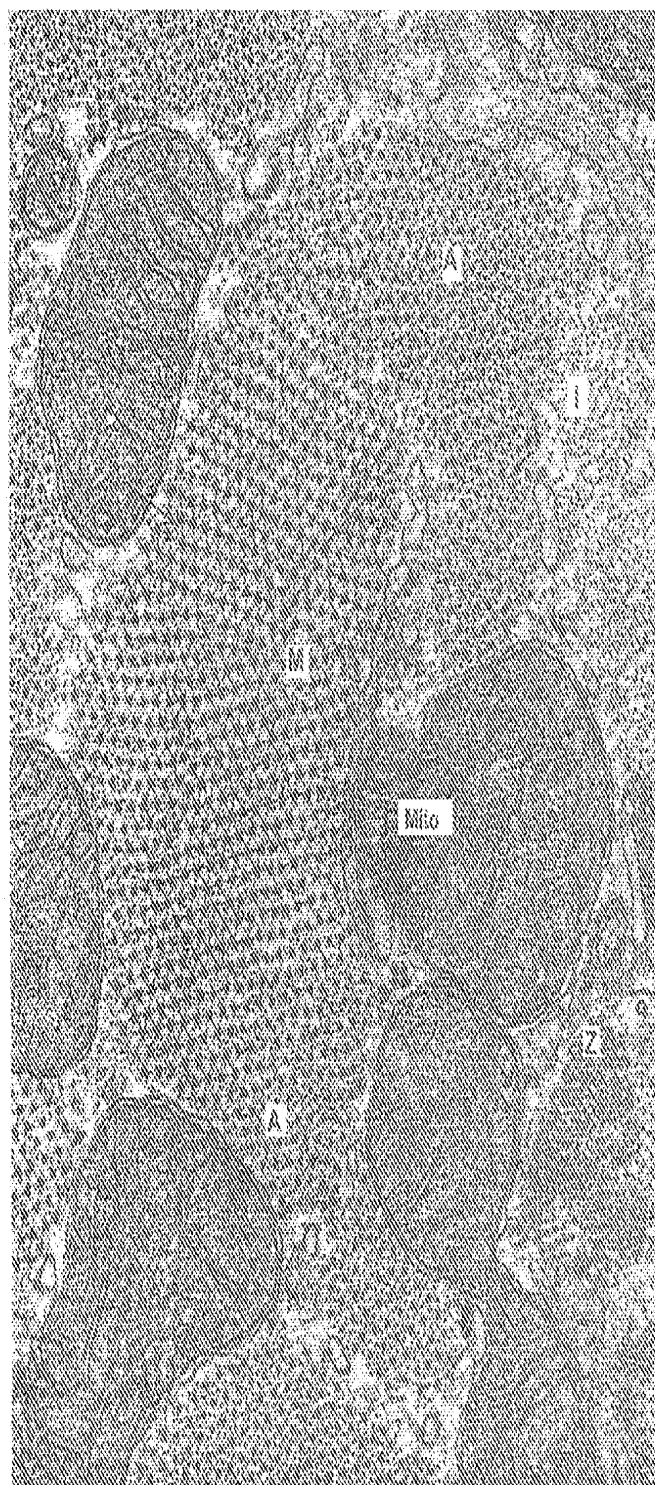
FIGURE 1-18 Cross section of a cat right ventricular papillary muscle showing mitochondria (Mito) and myofilaments cut at the level of the A-band (A), I-band (I), and M-band (M); in the latter, radial filaments link adjacent thick filaments (compare with Fig. 1-17). The Z-line (Z) appears as a dense network. (From McNutt and Fawcett, 1974.)

In the I-band, which lacks thick filaments, the thin filaments are less ordered. Radial cross-links, formed by myosin-binding protein C, link the thick filaments in a hexagonal array at the center of the A-band (Figs. 1-17 and 1-18).

The Plasma Membrane and Transverse Tubular System

The *plasma membrane* (*sarcolemma*), which separates the intracellular and extracellular spaces, forms *transverse tubules* (*t-system*) that extend into the cell (Fig. 1-14). These tubules, which contain extracellular fluid and open freely to the extracellular space, play a key role in excitation-contraction coupling by carrying action potentials deep into the cell (see Chapter 7). The plasma membrane contains channels, carriers, and pumps that regulate cell composition and function; receptors and enzymes that participate in cell signaling; and adhesion molecules that link cells to each other and to the extracellular matrix.

Intracellular Membrane Structures

Cardiac myocytes, like all eukaryotic cells, contain intracellular membrane-delimited organelles (Figs. 1-18–1-21). These include the *nucleus*, which contains the genetic material that determines cell structure, and *mitochondria*, which catalyze the oxidative reactions that generate most of the ATP used by the heart (Chapter 2). Mitochondria include an outer membrane that encircles these organelles and an inner membrane that contains infoldings called *cristae*. The latter contain key enzymes that participate in oxidative phosphorylation. When the heart is fixed under conditions that do not permit oxidative phosphorylation (e.g., low oxygen tension or low substrate concentration), the cristae appear as stacks of flat membrane sheets, whereas in hearts fixed when the mitochondria are carrying out oxidative phosphorylation, the cristae are angulated in an "energized" configuration. Phase contrast studies in living cardiac myocytes show that the mitochondria change constantly, enlarging and contracting, and branching and fusing with one another. Mitochondria contain circular DNA that is characteristic of prokaryotes; this reflects the origin of these organelles as micro-organisms that hundreds of millions of years ago crept into the cells of our progenitors (Margolis, 1970; Roger, 1999; Katz and Berger, 1999). In return for a nutrient-filled environment, these symbiotic invaders provide our hearts with a generous supply of ATP.

The *sarcoplasmic reticulum* (SR)—which takes up, stores, and releases the calcium that regulates contraction and relaxation (Chapter 7)—is a specialized form of the *endoplasmic reticulum* found in virtually every cell type. The endoplasmic reticulum in most cells includes a *rough endoplasmic reticulum* whose outer surface is studded with ribosomes that carry out protein synthesis, and *smooth endoplasmic reticulum* that participates in such processes as lipid metabolism and drug detoxification. In muscle, the major function of these internal membranes, often referred
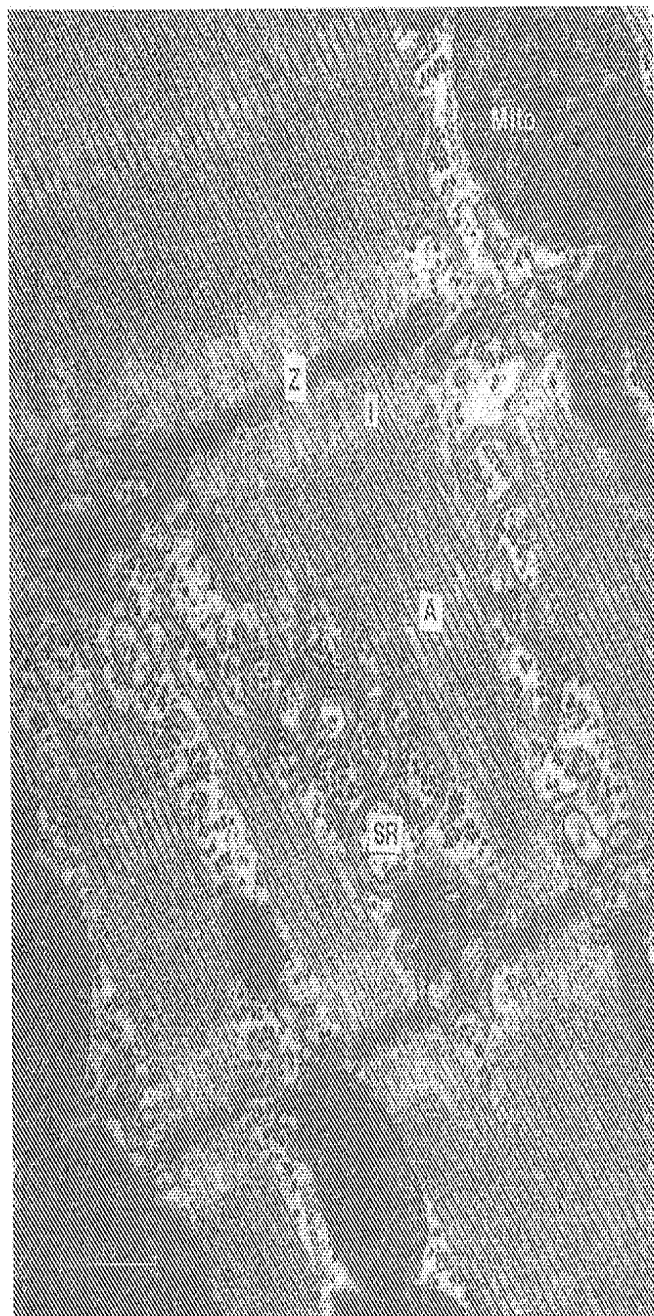
FIGURE 1-19 Electron micrograph of rat ventricular muscle showing the sarcotubular network (SR) in a "grazing" section overlying a sarcomere (center). The dark granules are glycogen. A faint linear structure, composed of two parallel lines, that crosses the sarcotubular network at the lower right is probably a microtubule. Mito, mitochondria; A, A-band; I, I-band; Z, Z-line. Scale bar = 1 μm. (Courtesy of Mrs. Judy Upshaw-Earley and Dr. Ernest Page.)

to as the *sarcoendoplasmic reticulum* (SERCA), is to regulate cytosolic calcium concentration.

The cardiac sarcoplasmic reticulum consists of two regions (Figs. 1-14 and 1-20). The *sarcotubular network*, which pumps calcium out of the cytosol, is a network of tubules that surrounds the myofilaments.
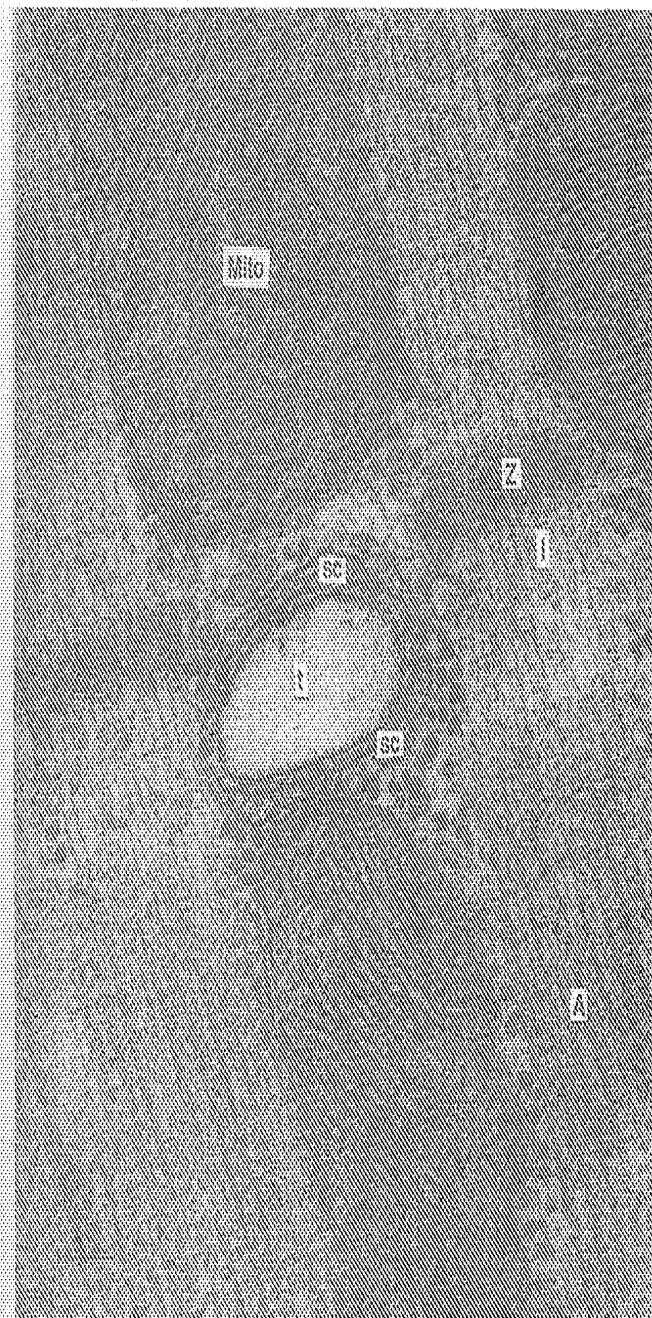
FIGURE 1-20 Cross section of dyad in rat ventricular muscle. The transverse tubular system (t), seen in cross section, lies between two subsarcolemmal cisternae (sc). Electron-dense feet (arrows) can be seen in the cytosol between the membranes of the t-tubule and subsarcolemmal cisterna. Mito, mitochondria; A, A-band; I, I-band; Z, Z-line. Scale bar = 0.1 μm. (Courtesy of Mrs. Judy Upshaw-Earley and Dr. Ernest Page.)

Subsarcolemmal cisternae, which release calcium from the sarcoplasmic reticulum into the cytosol in response to plasma membrane depolarization, are flattened structures that form composite structures with the plasma membrane. In the latter, called *dyads*, the sarcoplasmic reticulum and plasma membranes approach one another but do not fuse. The

FIGURE 1-21   Schematic diagram of a dyad, showing sarcoplasmic reticulum calcium release channels ("ryanodine receptors") adjacent to plasma membrane calcium channels ("dihydropyridine receptors") in the t-tubule. The former, which form the "feet," have a single opening into the cytosol and four openings into the lumen of the subsarcolemmal cisterna.

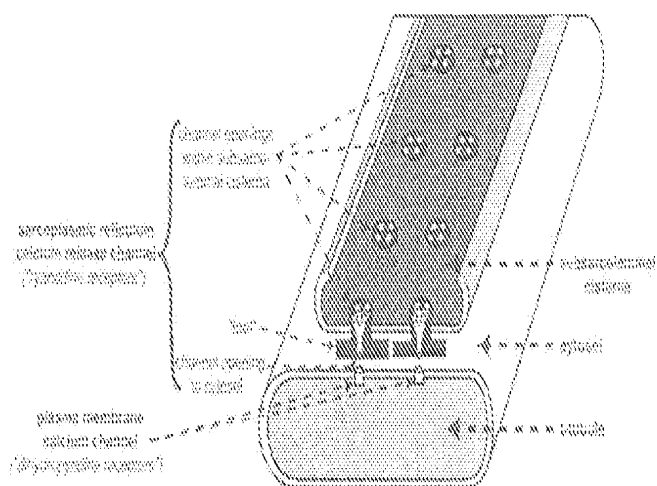narrow cytosolic space between these membranes contains huge electron-dense proteins, often called *feet* because they resemble the feet of a caterpillar (Franzini-Armstrong and Nunzi, 1983) (Figs. 1-20 and 1-21). These foot proteins are the calcium release channels of the sarcoplasmic reticulum (called *ryanodine receptors*) that open to initiate cardiac contraction by allowing calcium to flow out of the sarcoplasmic reticulum into the cytosol (Chapter 7). The sarcoplasmic reticulum calcium release channels differ from the L-type calcium channels (called *dihydropyridine receptors*) found in the plasma membrane.

The Cytoskeleton

Cells contain a network of filaments called the *cytoskeleton*, which maintains cellular architecture, forms mechanical linkages between cells and with the extracellular matrix, organizes enzymes that participate in integrated catalytic cycles, and maintains the proximity of membrane pumps and channels that regulate key ion fluxes. The cytoskeleton also plays an important role in cell signaling, such as allowing different types of cell deformation to initiate specific proliferative responses in response to various types of overload (see Chapter 5).

The cytoskeleton contains three fiber types: *microfilaments*, *microtubules*, and *intermediate filaments*. Microfilaments, which contain actin, are found in two structures: *sarcomeric actin filaments*, which are the thin filaments of the myofibrils described above, and *cortical actin filaments* that form a network beneath the plasma membrane. The latter link the contractile machinery to other cytoskeletal elements, anchor cells to each other, and connect the cytoskeleton to

the extracellular matrix. Cortical actin filaments interact with members of an extended family of myosin molecules (see Chapter 4) to transport membrane vesicles to and from the cell surface. Microtubules, which have a tubulin backbone, form networks that transport cell organelles and participate in cell division; specialized arrays of microtubules are instrumental in the movements of cilia and flagella. Microtubular transport is analogous to muscle contraction except that the filaments are polymers of *tubulin*, rather than actin, and the "motor proteins" are *kinesins* and *dyneins*, rather than myosin. The third type of cytoskeletal fiber, the intermediate filaments, are polymers of *desmin* that form very strong rivetlike structures called *desmosomes* that help maintain cell architecture, link cells to one another, and attach cells to the extracellular matrix. Unlike microfilaments and microtubules, intermediate filaments are not motile.

The Intercalated Disc

Specialized cell-to-cell junctions called *intercalated discs* (Fig. 1-22) form mechanical and electrical connections between cardiac myocytes (Table 1-3). (For a review, see Gallicano et al., 1998, Ferreira et al., 2003.) The mechanical linkages are provided by the *fascia adherens*, in which sarcomeric actin filaments are connected to networks of cytoskeletal actin filaments, and by *desmosomes* that connect intermediate filaments in adjacent cardiac myocytes. A third structure, the *gap junction*, contains large nonselective *connexin* channels that allow ions and other small molecules to diffuse freely between the cytosol of adjacent cells. By providing low-resistance connections between cells, gap junction channels allow electrical impulses to be conducted rapidly throughout the heart (see Chapter 15).

MEMBRANE STRUCTURE AND FUNCTION

Biological membranes can be viewed as barriers consisting of a central hydrophobic sheet that lies between two hydrophilic surfaces (Fig. 1-23). The barrier is provided by the lipid core, which is virtually impermeable to charged molecules, while charged phospholipid head groups on the surfaces interact with the aqueous media on the two sides of the membrane.

Membrane Lipids

Membranes contain a mixture of lipids, most of which are *amphipathic* in that they contain both hydrophilic (polar) and hydrophobic (apolar) moieties. Most membrane lipids are built upon glycerol, a 3-carbon sugar that is generally esterified to a hydrophilic "head group" and one or two hydrophobic fatty acyl chain "tails" (Fig. 1-24). Other membrane lipids include sphingolipids, in which the glycerol backbone is
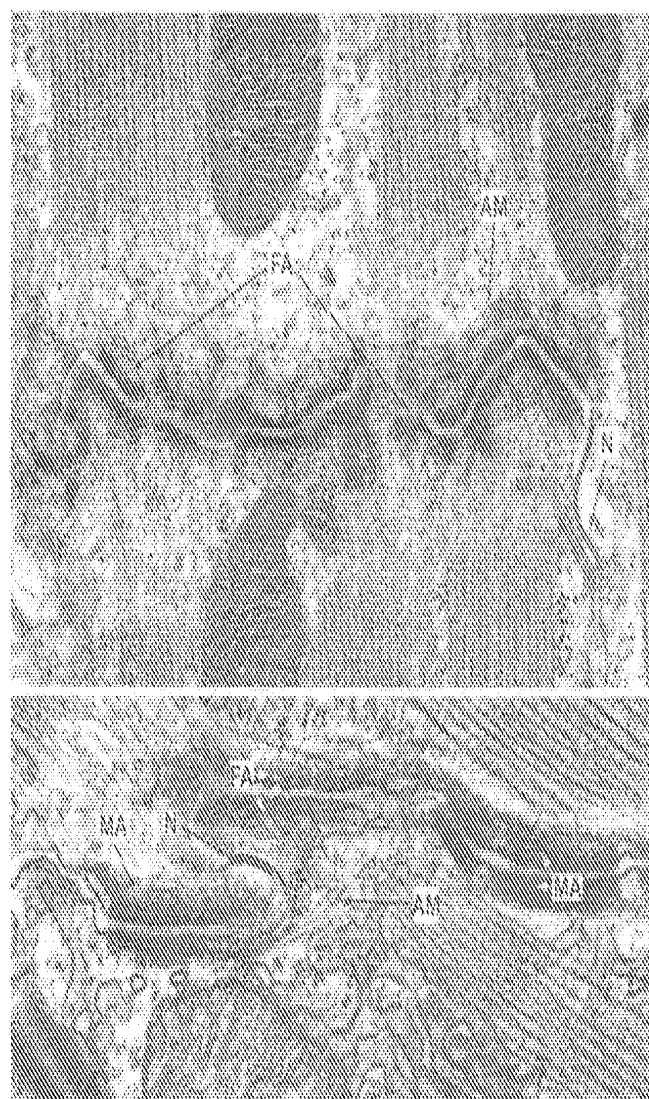
FIGURE 1-22 Electron micrographs of the intercalated disc. Top: transverse section of cat ventricular myocardium, showing insertions of sarcomeric actin microfilaments into the fascia adherens of the intercalated disc (FA), which is made up of cardiac actin microfilaments (AM). At the right, the intercalated disc continues as a nexus, or gap junction (N). Bottom, Oblique section of intercalated disc in mouse ventricular myocardium showing contractile actin neofilaments (AM), fascia adherens (FA), a nexus (N), and two desmosomes or macula adherens (MA). Modified from Bloom and Fawcett, 1974.

replaced by the 3-carbon amino acid serine. Most head groups contain charged anionic phosphate compounds, hence the term *phospholipid*. Cholesterol is found in the plasma membrane, where it reduces fluidity and "stiffens" the bilayer (Fig. 1-23).

Virtually all of the fatty acids in membrane lipids contain an even number of carbon atoms; in mammalian membrane, these are mainly palmitic and stearic acids (saturated C_{16} and C_{18}) and oleic, linoleic, and linolenic acids (unsaturated C_{18} fatty acids that contain 1, 2, and 3

TABLE 1-3 Cell-to-Cell Communication Across the Intercalated Disc

Structure	Type of Connection	Transmembrane Proteins	Cytoplasmic Proteins	Cellular Structure
Fascia adherens	mechanical	N-cadherin, β-1D integrin	β-catenin, plakoglobin, vinculin	microfilament (actin, α-actinin)
Desmosome	mechanical	desmoglein-2, desmocollin-2	desmoplakin, plakophilin, plakoglobin	intermediate filament (desmin)
Gap junction	electrical	connexin 43		ion channel

double bonds, respectively). Saturated fatty acids form relatively ordered regions in membrane bilayers, whereas regions made up of unsaturated fatty acids are more fluid (Klausner et al., 1980). Natural unsaturated membrane fatty acids are *cis*-isomers, in which the fatty acyl chains adjacent to the double bond are on the same side of the molecule. *Trans*-fatty acids, which occur in artificially hydrogenated fats, modify membrane
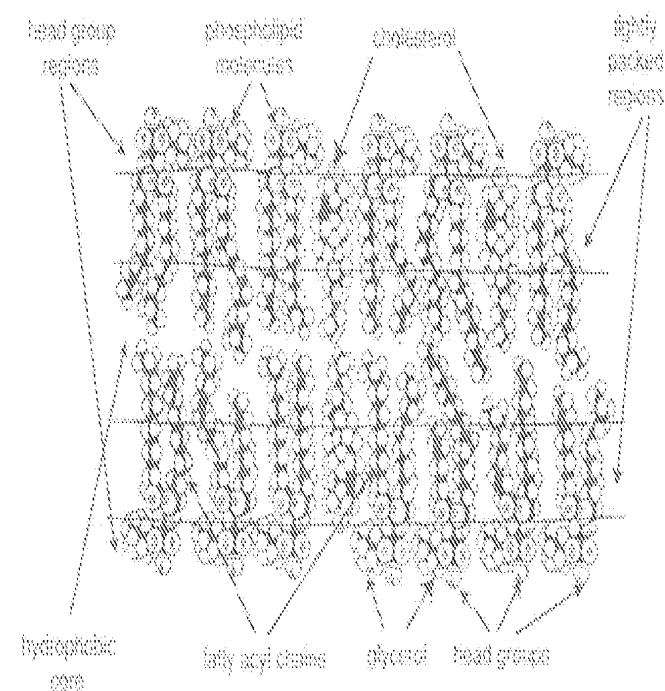

FIGURE 1-23 The membrane bilayer showing phospholipid molecules and cholesterol. The hydrophobic core, which is made up of uncharged nonpolar or hydrophobic fatty acyl chains and cholesterol, is lined by charged polar or hydrophilic "head groups." Tightly packed lipids lie between the head groups and hydrophobic core.
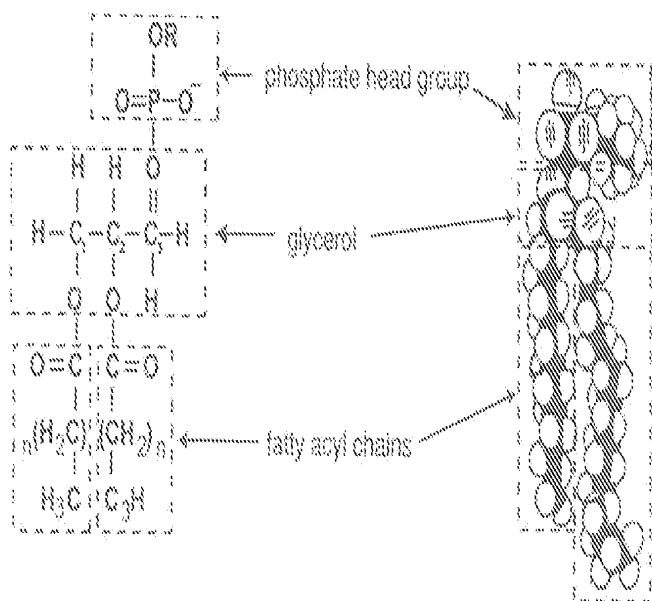
FIGURE 1-2A Structure of a phospholipid, oriented with the surface of the bilayer at the top, showing the glycerol "backbone" that is esterified to a head group and two fatty acyl chains. Left: Atomic structure. Right: Molecular model as shown in Fig. 1-23. The glycerol carbons are numbered 1, 2, and 3. The fatty acids in most phospholipids are esterified to carbons 1 and 2 and the head group, which can be linked to a variety of compounds (R), is esterified to carbon 3.

structure because the fatty acyl chains are on opposite (trans) sides of the molecule (Fig. 1-25).

Hydrolysis of membrane lipids by enzymes called *phospholipases* contributes to membrane damage in a number of diseases. *Phospholipases A₁* and *A₂* hydrolyze the ester bonds linking fatty acids to glycerol carbons 1 and 2, respectively (Fig. 1-26). *Phospholipase B* is a mixture of

FIGURE 1-26 Phospholipases A₁ and A₂ release the fatty acids esterified to glycerol at carbons 1 and 2, respectively, while phospholipases C and D release all or part of the head groups from glycerol carbon 3. When R represents inositol, phospholipase C releases a phosphosugar that provides the precursor for inositol phosphates; the remainder of the phospholipid that remains bound within the membrane, diacylglycerol, also serves as a second messenger.
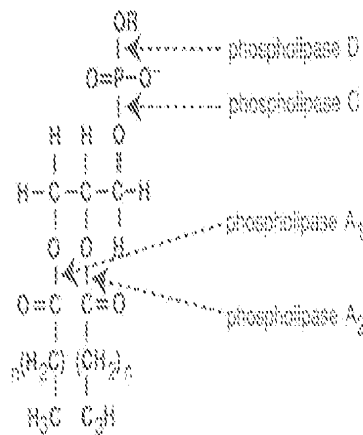
phospholipases A_1 and A_2 that hydrolyzes both of these ester bonds. *Phospholipase C* cleaves the phosphate head group from the glycerol "backbone," while *phospholipase D* removes organic structures from the head group, leaving the phosphatidic acid moiety attached to carbon 3 of glycerol.

Membrane lipids released by phospholipases often serve as signaling molecules (see Chapters 8 and 9). For example, hydrolysis of the membrane phospholipid *phosphatidylinositol 4,5-bisphosphate* by phospholipase C releases two messengers: *diacyl glycerol* (DAG) and *inositol triphosphate* (InsP₃). *Arachidonic* acid (unsaturated C_{20}) released from membrane phospholipids by phospholipase A_2 is the precursor of a family of extracellular messengers that includes *prostaglandins, thromboxanes,* and *leukotrienes,* while *myristic* (saturated C_{14}) and *palmitic* acids released into the cytosol can be covalently linked to proteins where they modify function (Resh, 1999; Chen and Manning, 2001; Farazi et al., 2001).

Membrane Proteins

Most of the important activities of biological membranes are mediated by *intrinsic membrane proteins* that are imbedded in one or both leaflets of the bilayer (Fig. 1-27). These proteins, which can make up more than one-half of the weight of a membrane, include receptors, enzymes, channels, carriers, pumps, and exchangers. The extracellular portions of plasma membrane proteins often contain covalently bound lipid (lipoproteins) or carbohydrate (glycoproteins).

The fluid nature of the lipid bilayer allows the membrane proteins to move in the plane of the bilayer, much as icebergs float in the sea. The lipids that surround the hydrophobic surfaces of membrane proteins, sometimes called the *boundary layer lipids* or *annulus,* play an important role in regulating the activity of these proteins (Katz and Messineo, 1981). Changes in this lipid environment have been implicated in the pathogenesis of clinical arrhythmias (Kang and Leaf, 1996; Leaf and Xiao, 2001;
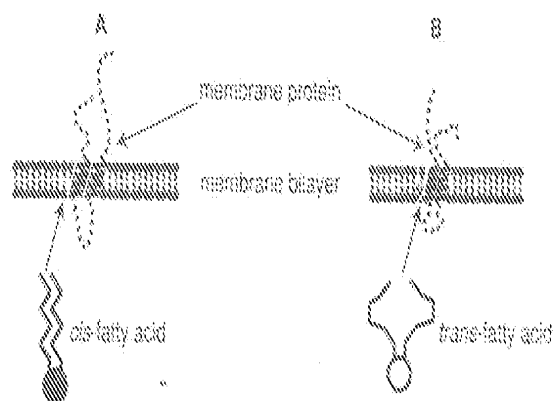
FIGURE 1-25 Schematic representation of phospholipids containing cis- (A) and trans- (B) unsaturated fatty acids. Because the conformations of the fatty acyl chains are different, trans-fatty acids can modify the conformation and function of membrane proteins.
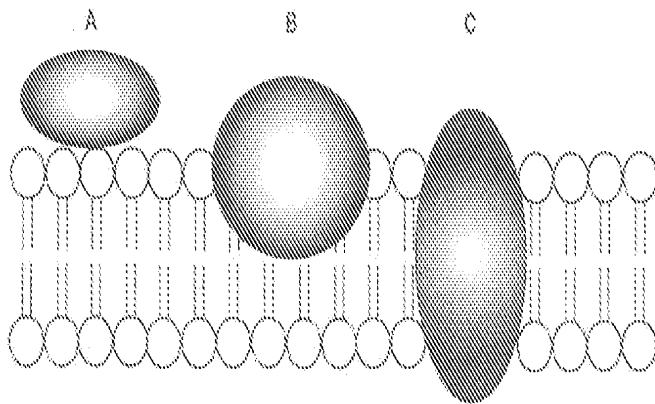
FIGURE 1-27 Membrane proteins (shaded) can be adsorbed to the membrane surface (A), incorporated into one leaflet of the bilayer (B), or can span the bilayer (C). Parts B and C represent intrinsic membrane proteins.

Katz, 2002). Many cardioactive drugs are amphipathic molecules that modify function when they enter the bilayer and interact with the hydrophobic surfaces of membrane proteins (Herbette and Mason, 1991).

Membrane Transport

Transport of materials across the membrane barrier can be effected by two fundamentally different mechanisms. The first, exemplified by the ion pumps and ion exchangers described in Chapter 7 and the ion channels discussed in Chapter 13, are generally highly selective for a given molecule. Not all membrane channels exhibit this specificity. For example, the gap junction channels in the intercalated disc allow a variety of molecules to move between neighboring cells, and anion channels in the sarcoplasmic reticulum allow several anions to cross this internal membrane.

An entirely different mechanism that transports large molecules across the plasma membrane occurs when parts of the membrane invaginate and then pinch off to form vesicles that move through the cytosol. An example is exocytosis, where intracellular membrane vesicles transport substances manufactured within cells to the cell surface where the vesicles fuse with the plasma membrane, which allows the substances to be released into the extracellular fluid. Bulk transport in the opposite direction occurs by endocytosis, in which molecules, often bound to a specific receptor, enter cells within vesicles formed by invagination of the plasma membrane. These transport processes are facilitated by "molecular motors" that are powered by interactions between cortical actin filaments and nonmuscle myosin and of tubulin with kinesins and dyneins (see above).

Endocytosis is effected by several mechanisms. In pinocytosis, vesicles formed from plasma membrane invaginations take up small amounts
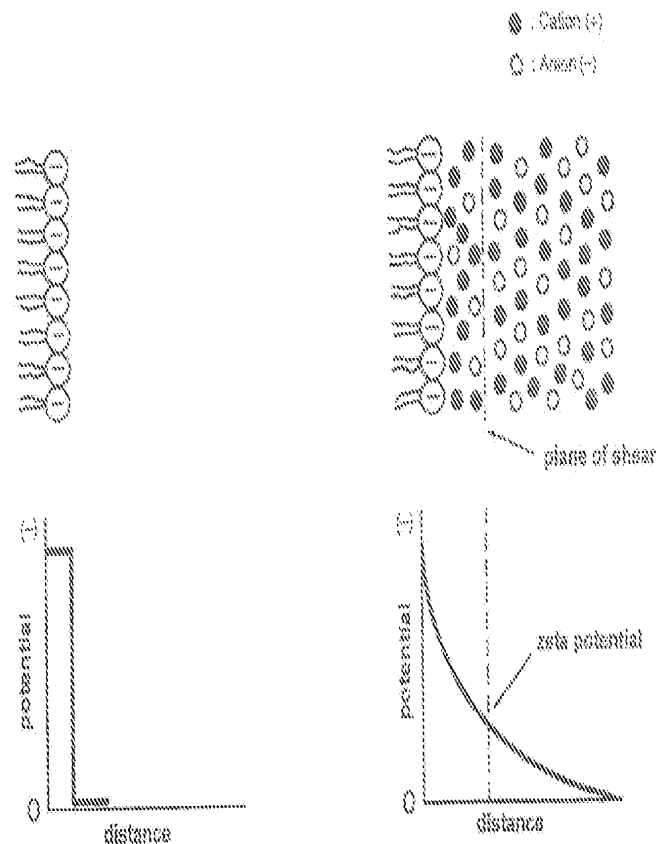
FIGURE 1-28 Distribution of electrical potential at the surface of a membrane composed of phospholipids with negatively charged head groups. Left: Surface charge falls sharply with increasing distance from the membrane when ions are absent in the surrounding medium. Right: When salts are included in the medium adjacent to the membrane, attraction of the cations to the anionic surface causes a more gradual fall in surface charge. Some of these cations remain associated with the membrane when it is moved through to the surrounding medium, giving rise to a "plane of shear" outside of which ions move freely. The potential at the plane of shear is the zeta potential.

of extracellular fluid, which is then transported into the cell. Receptor-mediated endocytosis occurs when selected molecules in the extracellular fluid (ligands) bind to specific receptors on the outer surface of the plasma membrane; the ligand-bound receptors then stimulate the adjacent plasma membrane to invaginate. These invaginations, which are called coated pits because their cytosolic surfaces are lined by proteins such as clathrin and caveolin, form sealed coated vesicles that contain the receptor-bound ligands. These vesicles then fuse with other intracellular vesicles called endosomes that can be transported within cells.

Surface Charge and Transmembrane Potential

Anionic phosphate moieties in the head groups of membrane lipids give rise to a negative surface charge that attracts cations in the

aqueous media toward the membrane surface. The result is a gradual change in surface potential as one moves away from the membrane (Fig. 1-28). The potential at the plane of shear when the membrane moves through the surrounding aqueous medium is called the *zeta potential*.

Biological membranes often separate regions of different electrical potential; cardiac Purkinje fibers, for example, have a potential difference across the resting plasma membrane of about 90 mV. Changes in the magnitude, and often the polarity, of this potential difference exert forces that modify the conformations of intrinsic membrane proteins such as *voltage-gated ion channels* (Chapter 13). Although the absolute potential differences across the plasma membrane are small, they create enormous electrical potential gradients because they occur across a very thin surface. A potential difference of -90 mV (-90×10^{-3} V viewed from within the cell) across the sarcolemma, which is approximately 30 Å (30×10^{-8} cm) thick, represents a potential *gradient* of $-300,000$ V/cm (90×10^{-3} V $\div$ 30×10^{-8} cm). During depolarization, this potential difference becomes $+30$ mV, so the gradient reverses to $+100,000$ V/cm. This means that the *change* in transmembrane potential gradient is about 400,000 V/cm! These large changes in potential gradient explain how what seem to be small changes in transmembrane potential generate powerful forces that can open and close ion channels.

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